（Format No. 13）

SUMMARY OF DOCTORAL THESIS

Name: Mostafa Abdelrahman

Title: Biochemical Analyses of Functional Metabolites in *Allium*: Prospective Strategies for Improving Crop Stress Tolerance

（ネギ属における機能性代謝物の生化学分析：作物ストレス耐性の改変に関する将来戦略）

Consumption of the *Allium* species as food and an ethnomedicine is mainly attributed, respectively, to its nutritional and functional properties, including antiasthmatic, antichlolesterolemic, and antimicrobial properties. The genus *Allium* is enormous (800 to 850 species) and is broadly spread over the northern hemisphere. A region of exceptionally wide genetic diversity stretches from the Mediterranean Basin to Central Asia. *Allium* species have adapted to diverse ecological niches, leading to the development of an astonishing number of different morphotypes. This is the main reason for the widely recognized difficulties in the taxonomy of *Allium.* The need for disease resistance materials in onion breeding has promoted studies of biosystematics relationships between cultivated and wild *Allium* species. The wild and local *Allium* species, such as *A. roylei* and shallot (*A. cepa* L. Aggregatum group), serve as a potential source for onion crop improvements in order to develop new varieties with some favorable characteristics, including health-enhancing qualities and disease resistance. In the present study, an approach for profiling targeted metabolites was adopted for a strong focus on saponin compounds as prospective chemically based genetic markers 1) for exploring an intraspecific variation within *Allium* species subg. Melanocrommyum and Nectaroscordium, 2) for isolating, purifying, and evaluating potential compounds related to *Fusarium* disease resistance from *A. nigrum*, 3) for isolating and purifying several saponin compounds from shallots and applying them as chemical marker for selecting *Fusarium* disease resistance within different shallots and bulb onion hybrid populations, as well as F_1, amphidiploid, and allotriploid lines of *A. roylei* and shallots, 4) for investigating the involvement of *A. roylei* metabolites in the phenotypic expression for disease resistance, and 5) for investigating the transcriptome and target metabolome variability in doubled haploids of *A. cepa* for prospective stress responsiveness.

The first part of this study revealed the profile of thin-layer chromatography and the remarkable antifungal activity against different *Fusarium* pathogens of the crude saponins extracted from *Allium* species subg. Melanocrommyum and Nectaroscordium. However, with the exception of *A. siculum*, which shows a distinguished ACSO composition and considerably higher amounts, very low contents of flavor precursors (cysteine sulfoxides, ACSOs) were found in these species. The promising saponin compounds in these subgenera can be used as chemical markers for disease resistance selection and chemotaxonomy of the genus *Allium.*

In the second part, a deep understanding of the metabolites' composition of *A. nigrum* was successfully gained through HPLC and spectral analyses. Phytochemical analyses of ACSOs, total polyphenols (TPs), and total saponins revealed quantitative variations within the different organs of *A. nigrum*. The excessive accumulation of ACSOs was detected in scaly bulbs (0.367 mg/g fw), of TPs in leaf blades (116.05 mg CE/100 g fw), and of saponins in roots (19.38 mg/g dw). The chromatographic characterization of *A. nigrum* root extract led us to isolate a spirostanol saponin. The structure was elucidated by spectroscopic analysis (2D NMR, FABMS, HR-ESI-MS). The structure of this compound was identified as 25(R,S)-5a-spirostan-2a,3b,6b-trio1-3-O-b-D-glucopyranosyl-(1,2)-O-[b-D-xylopyranosyl-(1,3)]-O-b-D-glucopyranosyl-(1,4)-b-D-galactopyranoside

or aginoside. The highest aginoside content, 2.9 mg/g dw, was detected in the root. The *in vitro* and *in vivo* antifungal activity of aginoside was evaluated for the first time against different phytopathogens, providing a new avenue for saponin application in the agrochemical industries.

The same pattern of research was carried out using shallots for plant material. The output of this part was accomplished by the isolation and identification of two important spirostanol saponin compounds, Alliospiroside A and Alliospiroside B. Among all of the compounds isolated, Alliospiroside A exhibited powerful antifungal activity against *Fusarium* pathogens, which indicates the importance of this compound for disease resistance. Quantitative and qualitative variations were observed in the TLC profile of shallot ('Chaing-Mai') and bulb onion hybrid populations (BC_1, BC_1S_1, and BC_1S_2). We clearly observed an intensive accumulation of Alliospiroside A in the resistance lines as compared with those of susceptible lines. Moreover, furostanol saponins can be a reservoir for the biosynthesis of spirotanol saponins, and the gene related to this biotransformation (beta-glucosidase) could be an important genetic marker for disease resistance selection.

To investigate the involvement of *A. roylei* metabolites in the plant's defenses, the contents of ACSOs, flavonols, polyphenols, ascorbic acid, and saponins in the various organs of this species were comprehensively analyzed. The root-basal stem, bulb, and leaf extracts exhibited 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with inhibition concentrations (IC_{50}) ranging from 0.649 to 0.757 mg/mL. The antimicrobial properties of the saponin and flavonoid crude extracts were evaluated. The saponin extracts demonstrated significant antifungal activity dependent on the applied concentration, and the growth inhibition rate of the tested fungal pathogens ranged from 1.07 to 47.76%. No appreciable antibacterial activity was recorded in the same sample. HPLC and spectral-based analyses together with histochemcial studies have provided important insight into the validity of saponins as a key component in plant protection.

Environmental stress conditions, such as drought, heat, salinity, or pathogen infection, can have a devastating impact on plant growth and yield, resulting in a mandate for stress-tolerant crop varieties. In this study, n intraspecific hybridization between a doubled haploid shallot (DHA) and doubled haploid bulb onion (DHC) was carried out to obtain the F_1 hybrid at first. The targeted metabolite profiling, using liquid chromatography/tandem-mass spectrometry (LC-MS/MS) integrated with transcriptional analysis of their relevant genes, provides insight into the metabolic and genomic architecture of DHA, DHC, and the F_1 hybrid. From a complete set of 113 targeted metabolites, 49 metabolites were found to be statistically different among the genotypes; 11 metabolites were characteristic for DHC, 10 for DHA, 14 for F_1, and 14 metabolites were mutual among the three genotypes. Several key genes and metabolites introgressed in abiotic stress responsiveness have been up-regulated in DHA and F_1 hybrids, as compared with DHC, even in non-stressed conditions. Principal component analysis (PCA) and Volcano plot analysis revealed that metabolic traits (*i.e*., amino acid, carbohydrate, flavonoid, and phospholipid biosynthesis) and their relevant genes were mostly over-expressed with the existence of shallot genome (s) in DHA and F_1, reflecting the adaptability to abiotic stress of these two genotypes as compared with that of DHC.

All of the results obtained create prospects for developing new varieties of *Allium* vegetables and carrying out further omics approaches, both in cultivated and wild species of this genus.